Sterols and Terpenoids from *Melia azedarach*

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Three new sterols (**1**−**3**) including an unprecedented ring A-seco natural product (**1**), five new terpenoids (**4**−**8**), and 15 known compounds were isolated from the bark of *Melia azedarach*. Their structures were elucidated by means of spectroscopic data, and the structure of **1** was confirmed by X-ray crystallography.

Melia azedarach Linn. (Meliaceae), widely distributed in southern districts of the Yellow River in China,[1] has been used as a biopesticide.[2] The bark of *M. azedarach* has been used as an insect repellent, for threadworm, and as a therapeutic medicine for tinea imbricata in the Chinese Pharmacopoeia.[3] The plant is known to contain sterols, terpenoids, protolimonoids, and limonoids and to show analgesic, anticancer, antiviral, antimalarial, antibacterial, and antifeedant activities.[4,5] Now we describe the isolation and structure elucidation of three new sterols (**1**−**3**), four new tirucallane triterpenoids (**4**−**7**), and a new tetranortriterpenoid (**8**) from an ethanol extract of *M. azedarach* bark.

Results and Discussion

Fractionation of a 90% ethanol extract of *M. azedarach* bark by repetitive chromatographic separation yielded eight new compounds (**1**−**8**). Compound **1** was obtained as colorless needles (MeOH) and possessed the molecular formula $C_{21}H_{30}O_5$ by HRESIMS, indicating seven degrees of unsaturation. The IR spectrum showed absorption peaks at 3432 (OH), 1729 (C═O), and 1631 cm^{-1} (C═C), respectively. UV absorption at λ_{max} 258 nm (log ε 3.79) was consistent with a five-membered α,β-unsaturated ketone chromophore.[6] The signals at δ_H 11.98 (2H, br s) in the ^{1}H NMR spectrum and corresponding signals at δ_C 174.4 (s) and 172.5 (s) in the ^{13}C NMR spectrum indicated the presence of two carboxyl groups. Signals of a ketone group (δ_C 207.7, s) and a trisubstituted double bond (δ_C 148.2, 129.0) were also presented. Therefore, **1** was deduced to be a ring-seco sterol on the basis of the three remaining degrees of unsaturation and the signals of three methyl groups and two high-fielded quaternary carbons in the ^{13}C NMR spectrum. In the HMBC spectrum, correlations of δ_C 172.5 (C-2) with δ_H 2.23 (H-1) and 0.76 (H-19) and of δ_C 174.4 (C-3) with δ_H 2.51 (H-4) suggested the C-2/C-3 bond was cleaved to form two carboxyl groups. Moreover, correlations of δ_C 148.2 (C-17) with δ_H 0.84 (H-18) and 1.98 (d, H-21) and of δ_C 207.7 (C-16) with δ_H 5.71 (q, H-20) and 2.04 (H-15) in the HMBC spectrum indicated that the ketone group at C-16 was conjugated with the double bond at C-17/C-20. The α-orientation of H-5 was suggested by correlation of δ_H 2.05 with δ_H 1.37 (H-9) and δ_H 1.28 (H-14) in the ROESY spectrum (Figure 1). Thus, compound **1** was elucidated as 2,3-seco-dicarboxylpregn-17-en-16-one. Since the ring A-seco pregnane was unusual, an X-ray diffraction study was performed to confirm the structure (Figure 2).

Compound **2** ($C_{21}H_{32}O_5$) was obtained as colorless needles. The IR spectrum showed absorptions at 3444 cm^{-1} (OH) and 1639 cm^{-1} (C═C). Signals of two high-field quaternary carbons and an *O*-methylene group (δ_C 63.0) in the ^{13}C NMR spectrum, along with a singlet methyl and a triplet methyl in the ^{1}H NMR spectrum, suggested that **2** was a pregnane derivative. The ^{13}C NMR spectrum also showed signals of a ketone group (δ_C 221.8), a trisubstituted double bond (δ_C 143.8 and 128.3), and three oxymethine groups (δ_C 68.1, 78.4, and 79.2) (Table 1). The *O*-methylene was assigned to C-18 by correlations of δ_C 63.0 with δ_H 1.78 (H-17) and of δ_C 47.4 (C-13) with δ_H 3.94, 3.54 (H-18) in the HMBC spectrum. Correlation of δ_C 221.8 with δ_H 1.78 (H-17) in the HMBC spectrum assigned C-16 as a ketone. The correlations of δ_H 3.20 (1H, dd, J = 9.6, 3.8 Hz) with δ_H 4.17 (1H, d, J = 3.8 Hz) and with δ_H 3.99 (1H, d, J = 9.6 Hz) in the ^{1}H−^{1}H COSY spectrum indicated three adjacent oxymethine groups. By comparing the chemical shifts and coupling constants with those of 2β,3β,4β-trihydroxypregnan-16-one and of 2α,3α,4β-trihydroxypregnan-16-one,[7] compound **2** was assumed to have a 2α,3β,4β-trihydroxy orientation, which was further supported by correlations of δ_H 3.99 (H-2) with δ_H 1.29 (H-19) in the ROESY spectrum (Figure 1). The double bond was assigned at C-5/C-6 on the basis of the correlations of δ_C 79.2 (C-4) and δ_C 33.2 (C-7) with δ_H 5.70 (H-6) and of δ_C 143.8 (C-5) with δ_H 4.17 (H-4) and δ_H 1.29 (H-19) in the HMBC spectrum. Thus, compound **2** was elucidated as 2α,3β,4β,18-tetrahydroxy-pregn-5-en-16-one.

Compound **3** had the molecular formula $C_{28}H_{46}O_4$ on the basis of the quasimolecular ion peak at m/z 469.3300 $[M + Na]^+$. The IR spectrum showed absorption peaks at 3415 cm^{-1} (OH) and 1639 cm^{-1} (C═C). The difference between compound **3** and 3β,16β,20-trihydroxyergosta-5,24(28)-diene[8] in the ^{13}C NMR spectrum (Table 1) was the presence of an oxymethine group in the former instead of a methylene group in the latter, indicating four OH groups in **3**. The additional OH was attached to C-22 on the basis of correlations of δ_H 3.82 (22-OH) with δ_H 1.76 and 2.19 (H-23) in the COSY spectrum and correlations of δ_C 78.6 (C-20) and δ_C 37.5 (C-23) with δ_H 3.82 (22-OH) in the HMBC spectrum (Figure 1). Unlike 3β,16β,20-trihydroxyergosta-5,24(28)-diene, the NOE correlations of δ_H 3.24 (H-3) with δ_H 0.94 (H-19) in the ROESY spectrum indicated the α-orientation of OH-3 in **3**. The structure was otherwise identical to that of 3β,16β,20-trihydroxyergosta-5,24(28)-diene by detailed analysis of the ^{1}H and ^{13}C NMR, HSQC, HMBC, ^{1}H−^{1}H COSY, and ROESY spectra of **3**. Thus, compound **3** was elucidated as 3α,16β,20,22-tetrahydroxyergosta-5,24(28)-diene.

Compound **4** had the molecular formula $C_{30}H_{44}O_4$ by HRESIMS, indicating nine degrees of unsaturation. The UV absorption bands at λ_{max} 244 nm (log ε 4.74) indicated an α,β-unsaturated ketone chromophore,[9] and IR absorptions were present at 3442 (OH), 1787 (γ-lactone), and 1642 cm^{-1} (conjugated double bond), respectively.[10] The ^{1}H NMR spectrum of **4** showed signals of two trisubstituted double bonds (δ_H 5.69, 5.09) and two geminal methyl groups (δ_H 1.61, 1.69) attached to an olefinic carbon (Table 2).

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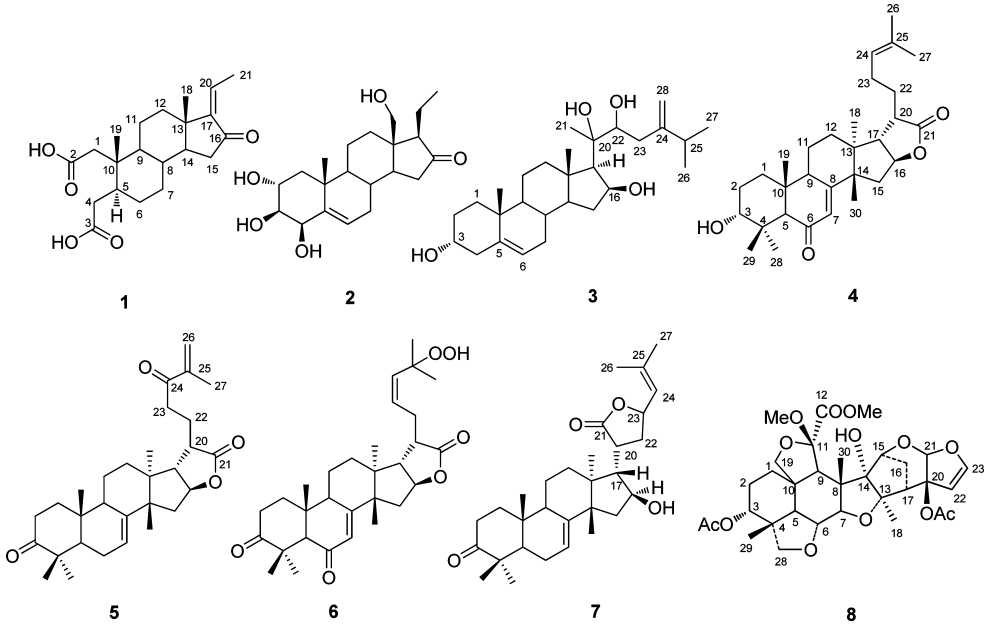
Chart 1



The ^{13}C NMR spectrum displayed signals of two carbonyl groups (δ_C 200.8, 180.1), two trisubstituted double bonds (δ_C 167.3, 132.9, 124.6, 123.2), and two oxymethine groups (δ_C 81.6, 76.6) (Table 3). The remaining degrees of unsaturation were ascribed to five rings. In the HMBC spectrum, correlations of δ_C 45.2 (C-20) with δ_H 4.15 (H-16) and of δ_C 180.1 (C-21) with δ_H 2.44 (H-20) (Figure 1) indicated the presence of a 20,16-olide moiety as in sendanolactone from the same species.[9] The difference between the two compounds was the presence of an OH at C-3 in **4** instead of the ketone group in sendanolactone, which was supported by correlations of δ_C 76.6 (C-3) with δ_H 1.25 (H-28) and δ_H 1.13 (H-29) in the HMBC spectrum (Figure 1). The broad singlet of H-3 suggested an α-orientation of the OH group. This assignment was supported by correlations of δ_H 3.36 (H-3) with δ_H 1.13 (H-29) and δ_H 1.27 (H-30) in the ROESY spectrum. The remaining structure was identical to that of sendanolactone by analysis of the ^1H and ^{13}C NMR, HSQC, HMBC, $^1H-^1H$ COSY, and ROESY spectra of **4**. Thus, compound **4** was 3α-hydroxytirucalla-7,24(25)-dien-6-oxo-21,16-olide.

Compound **5** had the molecular formula $C_{30}H_{42}O_4$ and IR absorptions of carbonyl groups at 1777 and 1707 cm^{-1}. The ^1H NMR spectrum exhibited six methyl groups, one of which was attached to an olefinic carbon, and three olefinic protons (Table 2). The ^{13}C NMR spectrum showed signals of three carbonyl groups (δ_C 216.4, 201.1, 180.5), a trisubstituted double bond (δ_C 118.5 143.3), and a terminal double bond (δ_C 125.1, 144.2) (Table 3). In the HMBC spectrum, correlations of δ_C 180.5 (C-21) with δ_H 1.84, 2.05 (H-22) and δ_H 2.49 (H-20) suggested a 20,16-olide moiety as in kulactone[10,11] (Figure 1). Correlations of δ_C 125.1 (C-26) with δ_H 1.86 (H-27) and of δ_C 201.1 (C-24) with δ_H 1.86 (H-27) and δ_H 6.01 (H-26a) in the HMBC spectrum indicated that the ketone group was conjugated with the terminal double bond. The chiral carbons in rings A−D and the lactone ring were identical to those of kulactone by analysis of the ^1H and ^{13}C NMR, HSQC, HMBC, $^1H-^1H$ COSY, and ROESY spectra of **5**. Thus, compound **5** was elucidated as tirucalla-7,25(26)-diene-3,24-dione-21,16-olide.

Compound **6** showed a quasimolecular ion peak at *m*/*z* 533.2677 $[M + Cl]^-$ in the negative HRESIMS, indicating the molecular formula $C_{30}H_{42}O_6$ and consistent with 10 degrees of unsaturation. UV absorptions at λ_{max} 244 nm (log ε 4.79) suggested an α,β-unsaturated ketone chromophore as in **4**. The ^1H NMR spectrum showed signals of three olefinic protons and a proton attached to an oxymethine carbon (Table 2). In comparison to sendanolactone,[10] the ^1H and ^{13}C NMR data of **4** and **6** were almost the same except

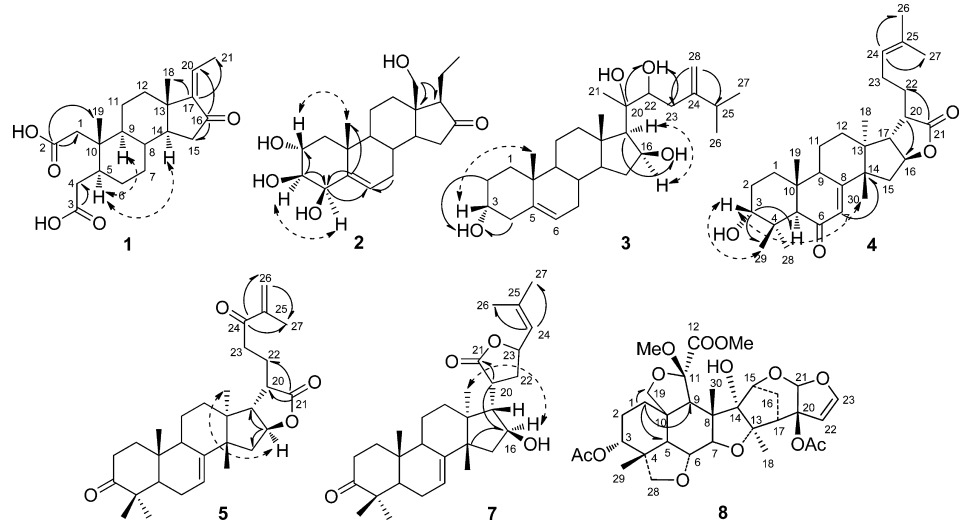


Figure 1. Key HMBC ($^{13}C \curvearrowright {}^1H$) and selected ROESY (dashed arrows) correlations for compounds **1**−**5**, **7**, and **8**.

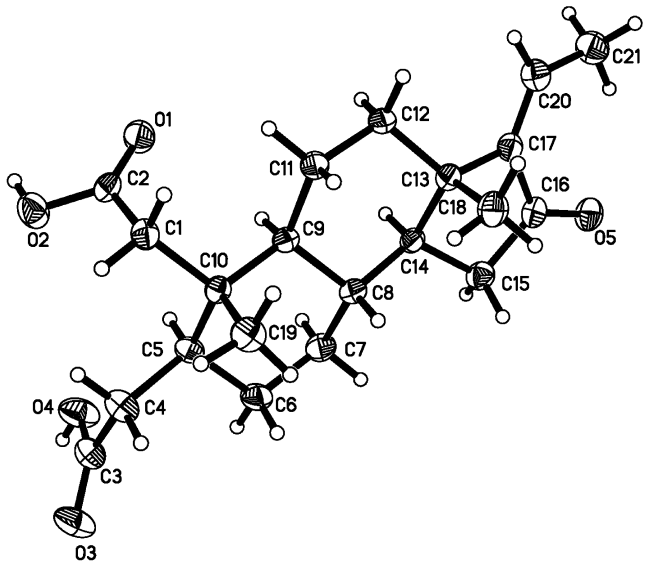


Figure 2. ORTEP diagram of **1**.

for signals of the side chain. A signal at δ_H 7.83 (1H, s) in the ^{1}H NMR spectrum and the corresponding quaternary carbon (δ_C 81.8) in the ^{13}C NMR spectrum, together with two remaining oxygen atoms in compound **6**, indicated that **6** must have a hydroperoxy group. This assignment was supported by the similarity of the spectroscopic data of **6** to those of meliastatin 3[12] regarding the side chain. Thus, the double bond in **6** was deduced to be at C-23/C-24, and the hydroperoxy group was placed at C-25 by comparison of its data with the two aforementioned compounds. Consequently, compound **6** was elucidated as 25-hydroperoxytirucalla-7,23(24)-dien-3,6-dion-21,16-olide.

Compound **7** ($C_{30}H_{44}O_4$) had IR absorption peaks at 3442 (OH), 1737 and 1705 (C=O), and 1630 cm^{-1} (C=C), respectively. The ^{13}C NMR spectrum exhibited signals of two ketone groups (δ_C 216.6, 181.7) and two trisubstituted double bonds (δ_C 144.3, 140.1, 121.7, 118.8). Considering the nine degrees of unsaturation and the aforementioned functionalities, **7** was deduced to have five rings. Comparing the spectroscopic data of **7** with those of 3,21-dioxotirucalla-7,24-dien-21,23-olide,[13] **7** showed an additional oxymethine signal instead of a methylene. Correlations of δ_H 4.28 (H-16) with δ_H 1.92 (H-17) and δ_H 2.10 (H-15a) in the ^{1}H−^{1}H COSY spectrum positioned the OH at C-16, which was supported by correlations of δ_C 48.9 (C-14) and δ_C 41.1 (C-20) with δ_H 4.28 (H-16) in the HMBC spectrum (Figure 1). Correlation of δ_H 4.28 (H-16) with δ_H 0.84 (H-18) in the ROESY spectrum indicated the β-orientation of 16-OH. Thus, compound **7** was elucidated as 16β-hydroxytirucalla-7,24(25)-dien-3-oxo-21,23-olide.

Compound **8** ($C_{32}H_{42}O_{13}$) showed IR absorption peaks for OH and carbonyl groups. The ^{1}H NMR, ^{13}C NMR, and DEPT spectra exhibited signals that were characteristic of meliacarpinin-type compounds (Tables 2 and 3).[14] Compared with 1-tigloyl-3,20-diacetylmethoxymeliacarpinin,[14] **8** was lacking signals of a tigloyl group. Instead, an additional methylene group in **8** corresponded to the 1-detigloyl derivative of 1-tigloyl-3,20-diacetylmethoxymeliacarpinin. This observation was supported by correlations of δ_C 33.2 (C-1) with δ_H 2.67 (H-5), δ_H 3.06 (H-9), and δ_H 3.96, 4.15 (H-19) in the HMBC spectrum. The coupling constants and chemical shifts of **8** at other protons and carbons were in good agreement with those of 1-tigloyl-3,20-diacetylmethoxymeliacarpinin.[14] Analysis of the ^{1}H and ^{13}C NMR, HSQC, HMBC, ^{1}H−^{1}H COSY, and ROESY spectra of **8** indicated that the remaining structure was identical to that of 1-tigloyl-3,20-diacetylmethoxymeliacarpinin. Thus, compound **8** was 3,20-diacetylmethoxymeliacarpinin.

Table 1. ^{1}H NMR Data of Compounds **1**,[a] **2**,[b] and **3**[a] (δ in ppm and J in Hz)

	1		**2**		**3**	
position	δ_H	δ_C, mult.	δ_H	δ_C, mult.	δ_H	δ_C, mult.
1	2.23, dd (10.4, 4.3)	40.5, CH_2	1.01, 2.02, m	46.6, CH_2	0.93, 2.11, m	37.2, CH_2
2		172.5, qC	3.99, t (9.6)	68.1, CH	1.35, 1.66, m	31.4, CH_2
3		174.4, qC	3.20, dd (9.6, 3.8)	78.4, CH	3.24, m	70.0, CH
4	2.51, d (14.9)	35.3, CH_2	4.17, d (3.8)	79.2, CH	2.12, m	42.8, CH_2
5	2.05, m	39.9, CH		143.8, qC		141.3, qC
6	1.23, 1.56, m	26.9, CH_2	5.70, t (2.0)	128.3, CH	5.25, d (5.6)	120.4, CH
7	1.39, 1.84, m	21.0, CH_2	1.79, 2.16, m	33.2, CH_2	1.42, 1.87, m	31.2, CH_2
8	1.42, m	33.9, CH	1.99, m	31.6, CH	1.22, m	30.6, CH
9	1.37, m	47.8, CH	1.23, m	52.1, CH	0.86, m	49.7, CH
10		39.1, qC		38.9, qC		36.1, qC
11	1.19, 1.81, m	35.4, CH_2	1.51−1.58, m	21.3, CH_2	1.45, m	20.1, CH_2
12	0.84, 1.53, m	31.0, CH_2	1.50, 2.02, m	36.1, CH_2	1.17, 2.03, m	40.0, CH_2
13		42.6, qC		47.4, qC		42.2, qC
14	1.28, m	49.2, CH	1.70, m	51.8, CH	0.84, m	53.8, CH
15	2.04, m	39.7, CH_2	2.13−2.18, m	39.8, CH_2	1.19, 1.84, m	37.7, CH_2
16		207.7, qC		221.8, qC	4.38, m	72.0, CH
17		148.2, qC	1.78, t (6.7)	64.2, CH	1.26, d (7.1)	56.2, CH
18	0.84, s	19.3, CH_3	3.54, d (11.5), 3.94, d (11.5)	63.0, CH_2	1.07, s	14.6, CH_3
19	0.76, s	15.5, CH_3	1.29, s	22.3, CH_3	0.94, s	19.2, CH_3
20	5.71, q	129.0, CH	1.51, 1.82, m	18.9, CH_2		78.6, qC
21	1.98, d (5.6)	13.6, CH_3	1.07, t (7.5)	14.2, CH_3	1.05, s	19.9, CH_3
22					3.83, s	73.8, CH
23					1.76, 2.19, m	37.5, CH_2
24						153.9, qC
25					2.30, m	32.5, CH
26					0.99, d (3.5)	21.9, CH_3
27					0.97, d (3.5)	21.7, CH_3
28					4.73, 4.76, br s	107.9, CH_2
−COOH	11.98					
3-OH					4.63, d (4.4)	
16-OH					5.95, d (3.4)	
20-OH					5.18, s	
22-OH					3.82, s	

[a] Spectra were recorded in DMSO-d_6. [b] Spectrum was recorded in CD_3OD.

Table 2. ^{1}H NMR Data of Compounds **4**−**8** ($CDCl_3$, δ in ppm and J in Hz)

position	4	5	6	7	8
1	1.35, m	1.43, 1.95, m	1.73, 1.99, m	1.41, 1.95, m	1.34, 1.58, m
2	1.70, 1.98, m	2.28, 2.79, m	2.35, 2.77, m	2.08, 2.73, m	1.78−1.86, m
3	3.36, br s				4.91, t (2.6)
5	2.57, s	1.73, m	2.46, s	1.68, m	2.67, d (12.8)
6		2.14, m		2.09, m	3.89, dd (12.6, 2.8)
7	5.69, br s	5.33, t (3.0)	5.80, br s	5.33, t (2.8)	4.26, d (2.7)
9	3.04, m	2.49, dd (12.7, 6.3)	2.96, m	2.28, m	3.06, s
11	1.60, 1.99, m	1.58−1.71, m	1.77, 1.96, m	1.58−1.62, m	
12	1.81, 1.98, m	1.74, m	1.69, 1.88, m	1.42, 1.79, m	
15	1.75, 2.33, m	1.72, m	1.85, 2.38, m	1.75, 2.10, m	4.14, s
16	4.15, ddd (10.2, 10.2, 7.8)	4.16, ddd (10.8, 10.2, 7.7)	4.22, ddd (11.8, 10.3, 7.7)	4.28, dd (8.8, 5.5)	1.82, 2.06, m
17	2.14, m	2.14, m	2.19, m	1.92, m	2.97, d (5.9)
18	0.98, s	0.96, s	0.99, s	0.84, s	1.37, s
19	0.87, s	1.02, s	1.12, s	0.99, s	3.96, d (8.5); 4.15, d (8.5)
20	2.44, m	2.49, m	2.45, m	2.77, m	
21					5.67, s
22	1.51, m	1.84, 2.05, m	2.47, m	2.10−2.19, m	5.41, d (3.0)
23	2.05, 2.13, m	2.95, t (5.4)	5.68, ddd (15.8, 7.9, 6.5)	5.25, m	6.42, d (3.0)
24	5.09, m		5.64, br, t (15.8, 5.7)	5.29, d (3.0)	
26	1.61, s	6.01, 5.79, br s	1.32, s	1.74, s	
27	1.69, s	1.86, br s	1.34, s	1.72, s	
28	1.25, s	1.04, s	1.37, s	1.24, s	3.45 (d, 7.5); 3.51 (d, 7.5)
29	1.13, s	1.11, s	1.38, s	1.09, s	0.97, s
30	1.27, s	1.24, s	1.36, s	1.01, s	1.49, s
25-OOH			7.83, s		
11-OCH_3					3.42, s
12-OCH_3					3.82, s
CH_3CO−					2.09, s
CH_3CO−					2.10, s

Table 3. ^{13}C NMR Data of Compounds **4**−**8** ($CDCl_3$, δ in ppm and J in Hz)

position	4	5	6	7	8
1	31.0, CH_2	38.2, CH_2	37.3, CH_2	38.4, CH_2	33.2, CH_2
2	24.3, CH_2	34.8, CH_2	33.9, CH_2	34.8, CH_2	24.8, CH_2
3	76.6, CH	216.4, qC	214.5, qC	216.6, qC	71.1, CH
4	39.3, qC	47.8, qC	47.1, qC	47.9, qC	42.3, qC
5	60.7, CH	52.5, CH	65.7, CH	52.3, CH	39.3, CH
6	200.8, qC	24.3, CH_2	197.9, qC	24.2, CH_2	71.7, CH
7	124.6, CH	118.5, CH	124.6, CH	118.8, CH	83.3, CH
8	167.3, qC	143.3, qC	167.6, qC	144.3, qC	51.9, qC
9	49.7, CH	47.7, CH	49.2, CH	47.8, CH	54.5, CH
10	44.2, qC	35.4, qC	43.8, qC	35.0, qC	46.1, qC
11	16.4, CH_2	16.7, CH_2	16.5, CH_2	17.6, CH_2	106.5, qC
12	29.0, CH_2	29.2, CH_2	31.5, CH_2	32.0, CH_2	170.0, qC
13	39.3, qC	39.5, qC	39.2, qC	45.8, qC	93.3, qC
14	56.0, qC	55.0, qC	56.1, qC	48.9, qC	92.6, qC
15	34.8, CH_2	35.6, CH_2	34.8, CH_2	43.9, CH_2	81.9, CH
16	81.6, CH	82.5, CH	81.6, CH	76.9, CH	28.9, CH_2
17	57.7, CH	57.9, CH	56.2, CH	57.4, CH	48.0, CH
18	21.3, CH_3	21.5, CH_3	21.4, CH_3	23.1, CH_3	25.7, CH_3
19	14.0, CH_3	12.4, CH_3	13.5, CH_3	12.7, CH_3	71.0, CH_2
20	45.2, CH	44.7, CH	45.4, CH	41.1, CH	91.7, qC
21	180.1, qC	180.5, qC	179.2, qC	181.7, qC	105.9, CH
22	29.2, CH_2	23.8, CH_2	28.5, CH_2	34.7, CH_2	105.5, CH
23	26.0, CH_2	34.7, CH_2	126.9, CH	76.0, CH	146.7, CH
24	123.2, CH	201.1, qC	137.2, CH	121.7, CH	
25	132.9, qC	144.2, qC	81.8, qC	140.1, qC	
26	17.9, CH_3	125.1, CH_2	24.5, CH_3	25.7, CH_3	
27	25.7, CH_3	17.6, CH_3	24.1, CH_3	18.3, CH_3	
28	27.9, CH_3	24.4, CH_3	25.1, CH_3	27.7, CH_3	76.0, CH_2
29	21.4, CH_3	21.4, CH_3	21.6, CH_3	21.5, CH_3	18.4, CH_3
30	29.7, CH_3	32.2, CH_3	29.6, CH_3	24.4, CH_3	17.4, CH_3
11-OCH_3					52.3, CH_3
12-OCH_3					52.7, CH_3
$OCOCH_3$					170.5, qC
$OCOCH_3$					171.5, qC
$OCOCH_3$					21.0, CH_3
$OCOCH_3$					21.3, CH_3

Fifteen known compounds were also isolated and identified as meliastatin 3,[12] kulonic acid,[15] kulactone,[10,11] sendanolactone,[9,10] toosendanone A,[16] dubione B,[12] 24-methylenecycloartenone,[17] meliavolin,[18] 12β,20(*S*)-dihydroxydammar-24-en-3-one,[19] dammarendiol II 3-*O*-caffeate,[20] toosendanin,[21] 1-tigloyl-3,20-diacetyl-11-methoxymeliacarpinin,[14] 2β,3β,4β-trihydroxypregn-16-one,[7] 3β-hydroxypregn-5,17(20)-dien-16-one,[6] and 5α,8α-epidioxyergosta-6,22-dien-3β-ol[22] by comparison of their physical and spectroscopic data with those published in the literature.

Experimental Section

General Experimental Procedures. Melting points were obtained on an X-4 micro melting point apparatus and are uncorrected. Optical rotations were recorded on a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer, and IR spectra were measured on a Tenor 27 spectrophotometer using KBr pellets. NMR spectra were acquired on Bruker DRX-500 or AV-400 spectrometers with TMS as the internal standard. Mass spectra were obtained on a VG Autospec-3000 spectrometer or an API QSTAR Pulsar 1 spectrometer. Column chromatography (CC) was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Ltd., Qingdao, P. R. China), RP-18 gel (40−65 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Ltd., Sweden). Fractions were monitored by TLC, and spots were visualized by heating the silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. Bark of *M. azedarach* was collected in Kunming, Yunnan Province, P. R. China, in October 2007. The sample was identified by Dr. Chun-Xia Zeng. A voucher specimen (Luo 071012) has been deposited with the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. An EtOH extract of dried bark of *M. azedarach* (30 kg) was concentrated, and the aqueous solution was partitioned with EtOAc. The EtOAc extract (820 g) was subjected to silica gel CC eluted with $CHCl_3$−Me_2CO (from 1:0 to 0:1) to give seven fractions (1−7). Fraction 2 (80 g), subjected to silica gel CC eluted with petroleum ether−Me_2CO from 20:1 to 8:1, gave four subfractions (2a−2d). Subfraction 2a (10 g) was separated on a RP-18 column (MeOH−H_2O, 6:1), followed by silica gel CC eluting with petroleum ether−EtOAc (5:1), to yield **5** (5.0 mg). Fraction 3 (88.0 g) was subjected to silica gel CC eluted with petroleum ether−Me_2CO from 10:1 to 4:1, yielding five subfractions (3a−3e). Separation of 3a (6.5 g) and 3c (7.8 g) on RP-18 eluted with MeOH−H_2O from 8:1 to 4:1 yielded **4** (5.8 mg) and **7** (42.2 mg), respectively. Subfraction 3d (9.8 g) was separated on silica gel eluted with $CHCl_3$−Me_2CO (9:1) to give **3** (15.0 mg). Fraction 4 (9.8 g) was subjected to RP-18 CC eluted with MeOH−H_2O (3:1), leading to **8** (98.5 mg). Fraction 7 (50 g) was subjected to silica gel CC eluted with $CHCl_3$−MeOH (from

15:1 to 5:1), followed by the RP-18 CC (MeOH−H_2O from 3:1 to 1:1) and Sephadex LH-20 (MeOH), yielding **6** (2.5 mg), **1** (15.0 mg), and **2** (11.1 mg).

2,3-Seco-dicarboxylpregn-17-en-16-one (1): colorless needles (MeOH); mp 254−255 °C; $[\alpha]_D^{20}$ −78.1 (*c* 0.29, DMSO); UV (DMSO) λ_{max} (log ε) 258 (3.79) nm; IR (KBr) ν_{max} 3432, 1729, 1631 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) data, see Table 3; HRESIMS: *m/z* 361.2016 $[M - H]^+$ (calcd for $C_{21}H_{29}O_5$, 361.2014).

2α,3β,4β,18-Tetrahydroxypregn-5-en-16-one (2): colorless needles (MeOH); mp 183−184 °C; $[\alpha]_D^{20}$ −71.8 (*c* 0.28, MeOH); IR (KBr) ν_{max} 3444, 1639 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) data, see Table 3; HRESIMS *m/z* 363.2158 $[M - H]^+$ (calcd for $C_{21}H_{31}O_5$, 363.2171).

3α,16β,20,22-Tetrahydroxyergosta-5,24(28)-diene (3): colorless needles (MeOH); mp 242−243 °C; IR (KBr) ν_{max} 3415, 1639 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) data, see Table 3; HRESIMS *m/z* 469.3300 (calcd for $C_{28}H_{46}O_4Na$, 469.3293).

3α-Hydroxytirucalla-7,24(25)-dien-6-oxo-21,16-olide (4): colorless needles (MeOH); mp 203−204 °C; $[\alpha]_D^{20}$ −11.1 (*c* 0.21, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 244 (4.74) nm; IR (KBr) ν_{max} 3442, 1787, 1659, 1642 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 491.3138 (calcd for $C_{30}H_{44}O_4Na$, 491.3137).

Tirucalla-7,25(26)-diene-3,24-dion-21,16-olide (5): colorless prisms (MeOH); mp 216−218 °C; $[\alpha]_D^{20}$ −48.2 (*c* 0.52, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.56) nm; IR (KBr) ν_{max} 1777, 1707 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 489.2965 (calcd for $C_{30}H_{42}O_4Na$, 489.2980).

25-Hydroperoxytirucalla-7,23(24)-diene-3,6-dion-21,16-olide (6): colorless prisms (Me_2CO); mp 197−198 °C; $[\alpha]_D^{20}$ −42.1 (*c* 0.58, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 244 (4.79) nm; IR (KBr) ν_{max} 3439, 1776, 1655 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 533.2677 (calcd for $C_{30}H_{42}O_6Cl$, 533.2669).

16β-Hydroxytirucalla-7,24(25)-diene-3-oxo-21,23-olide (7): colorless needles (MeOH); mp 233−234 °C; $[\alpha]_D^{20}$ −80.5 (*c* 0.31, $CHCl_3$); IR (KBr) ν_{max} 3442, 1737, 1705, 1630 cm^{-1}; ^1H NMR 500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 491.3134 (calcd for $C_{30}H_{44}O_4Na$, 491.3137).

3,20-Diacetoxy-11-methoxymeliacarpinin (8): colorless needles (MeOH); mp 249−251 °C; $[\alpha]_D^{20}$ −11.9 (*c* 0.53, $CHCl_3$); IR (KBr) ν_{max} 3486, 1748, 1612 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 657.2522 (calcd for $C_{32}H_{42}O_{13}Na$, 657.2523).

X-ray crystallographic analysis of 2,3-seco-dicarboxylpregn-17-en-16-one (1): $C_{21}H_{30}O_5$, $M = 362.45$; monoclinic, space group $P2_1$; $a = 7.3394(12)$ Å, $b = 12.818(2)$ Å, $c = 20.414(3)$ Å, $\alpha = \beta = \gamma = 90.00°$, $V = 1920.4(5)$ $Å^3$, $Z = 4$, $T = 298(2)$ K, $d = 1.254$ g/cm^3, $\lambda = 0.71073$ Å, $R_1 = 0.0617$ for 1267 observations with $I > 2\sigma(I)$, $wR_2 = 0.1098$ for all data. Crystallographic data for **1** have been deposited at Cambridge Crystallographic Data Center (deposition no. 747980).

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Supporting Information Available: The 1D, 2D NMR and MS spectra of new compounds **1**−**8** and crystal data for **1** are available free of charge via the Internet at http://pubs.acs.org.

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